Pseudoalteromonas rhizosphaerae sp. nov., a novel plant growth-promoting bacterium with potential use in phytoremediation.

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Abstract

Strain RA15^T was isolated from the rhizosphere of the halophyte plant *Arthrocnemum macrostachyum* growing in the Odiel marshes (Huelva, Spain). RA15^T cells were Gram staining-negative, non-spore forming, aerobic rods and formed cream-coloured, opaque, mucoid, viscous, convex, irregular colonies with an undulate margin. Optimal growth conditions were observed on TSA plates supplemented with 2.5% NaCl (w/v) at pH 7.0 and 28ºC, although it was able to grow between 4ºC-32ºC and pH values of 5.0-9.0. NaCl range tolerated was from 0% to 15%. The major respiratory quinone was Q8 but Q9 was also present. The most abundant fatty acids were Summed Feature 3 ($C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c), $C_{17:1}$ ω8c, and $C_{16:0}$ and the polar lipids profile showed phosphatidylglycerol and phosphatidylethanolamine as the most representatives. Phylogenetic analyses confirmed the well-supported affiliation of strain RA15^T within the genus *Pseudoalteromonas*, close to the type strains of *P. neustonica*, *P. prydzensis,* and *P. mariniglutinosa*. Comparative phylogenetic and phenotypic studies between the strain RA15^T and its closest related species suggest that RA15^T could be a new representative of the genus *Pseudoalteromonas*, for which the name *Pseudoalteromonas rhizosphaerae* sp. nov. is proposed. The type strain is RA15^T (=CECT 9079^T=LMG 29860^T). RA15^T whole genome has 5.3 Mb and G+C content is 40.4%.

Keywords: *Arthrocnemum macrostachyum*, Heavy metals, Odiel marshes, rhizosphere, Nitrogen fixation.

Pseudoalteromonas [1] is the type genus of the family *Pseudoalteromonadaceae* [2]. It is currently shaped by 47 species [3] characterized by presenting Gram staining-negative, motile, non-spore forming, aerobic, oxidase positive rods [4] requiring Na+ ion for growth [5] whose type species is *Pseudoalteromonas haloplanktis* [1,4,6]. Hitherto, all species described into the genus *Pseudoalteromonas* were isolated from marine environments, mainly seamount [7], sea water [4,8,9,10], and tidal flat [11], and as hosts of marine organisms [4,12,13,14].

Strain RA15^T was isolated from the rhizosphere of *Arthrocnemum macrostachyum* plants growing in the Odiel marshes (Huelva, Spain) [15]. 16S rRNA gene sequence analysis showed that it clustered within the genus *Pseudoalteromonas* with a 97.6% of similarity to the type strain of *Pseudoalteromonas prydzensis* [15]. It hydrolyses some substrates like starch, casein, tween 80, DNA, chitin and pectin [15]. Furthermore, strain RA15^T demonstrated ability to grow in high concentrations of heavy metals reaching values of 12 mM of As and 3 mM of Cu [15]. It also presented several plant growth-promoting (PGP) properties such as production of auxins and siderophores and nitrogen fixation, observing such traits even in presence of heavy metals, conditions under which higher expression of PGP traits were favored [15]. Likewise, strain RA15^T, as part of a bacterial consortium, improved the seed germination and the capacity of plants of *A. macrostachyum* to accumulate heavy metals in their roots [15,16].

This report aims the elucidation of the taxonomic status of the strain RA15^T, a strain showing biotechnological potential in the phytostabilisation of heavy metals polluted soils, in the genus *Pseudoalteromonas* following a polyphasic approach.

RA15^T was isolated from the rhizosphere of *A. macrostachyum* from the Odiel marshes (37º 13'N - 6º 57'O) as described in the work of Navarro-Torre *et al.* [15]. Succinctly, rhizosphere samples were mixed with sterile saline solution (0.9% (w/v)) and then shaken for 5 minutes. The suspension was plated on Tryptic Soy Agar (TSA) plates supplemented with 2.25% NaCl (w/v) (salt concentration present in Odiel marshes) and incubated for 72 h at 28ºC. Different colonies were isolated according to morphology and colour criteria and sub-cultured. Pure cultures were preserved in 15% glycerol at -80ºC.

Growth conditions were determined incubating the strain on TSA 2.25% NaCl (w/v). The range of temperature was tested at 4, 15, 20, 25, 28, 30, 32, 37 and 45ºC for 6 days. The

range of pH was assessed at 5.0, 6.0, 7.0, 8.0 and 9.0 for 6 days. pH values were adjusted with citrate-phosphate buffers (0.1M citric acid and 0.2M dibasic sodium phosphate) and Tris-HCl buffer (0.1M Tris (hydroxymethyl) aminomethane and 0.1M HCl). The growth in presence of NaCl was performed on mTGE (membrane Tryptone Glucose Extract) agar medium [17] from 0% to 30% (w/v) for 6 days at 28ºC. Growth under anaerobic conditions was carried out on semisolid TSA tubes containing 2.5% NaCl (w/v) and sealed with a first layer of 2% agar (w/v) and a second layer of paraffin and incubated for 10 days at 28ºC [18]. In addition, the bacterial growth in different media was determined on Marine Agar (MA) and the selective media Cetrimide agar and MacConkey agar, both supplemented with 2.5% NaCl (w/v), at 28ºC for 48h.

Colony appearance was studied on TSA 2.5% NaCl (w/v) plates after 24 h at 28ºC using a stereoscopic microscope (Olympus SZ61). The colony colour was determined using the colour chart RAL D2 Design. Cell morphology was studied using an optical microscope with 100X objective (Olympus CX41) after Gram staining [19]. Moreover cells of RA15^T were fixed with 2% uranyl acetate and morphology was observed using a transmission electron microscopy (Zeiss Libra 120). Motility was tested by incubating the strain in Tryptic Soy Broth (TSB) supplemented with 2.5% NaCl (w/v) at 28ºC for 30 min [15]. After that, a drop from the culture was observed under optical microscope with 40X objective.

To determine the catalase activity, a drop of 3% H_2O_2 was added to bacterial biomass. The test was considered positive if the reaction produced bubbles. For the oxidase activity, 1% N, N, N', N'-tetramethyl-p-phenylenediamine reagent (Becton, Dickinson and Company, Mexico) was added to bacterial biomass. When it turned blue in 10-15 seconds, the test was considered positive.

Biochemical characteristics were studied using API 20NE, API 20Strep, and API ZYM galleries (bioMérieux, France) according to the manufacturer's instructions. On the other hand, GEN III Microplates (BIOLOG Inc., Hayward, CA, United States) were used to determine the oxidation of carbon and nitrogen sources and the sensitivity to some inhibitory compounds. For the microplates inoculation, strain RA15^T was resuspended in a viscous inoculating fluid (IF) A supplemented with 2.5% NaCl (w/v) with a final transmittance of 95% and then microplates were inoculated. Microplates were incubated in an Omnilog device (BIOLOG Inc., Hayward, CA, United States) for 3 days at 30ºC. Results were analysed with the opm package for R [20, 21] v.1.3.72. In parallel, the same

protocol was carried out with the reference strains *Pseudoalteromonas prydzensis* DSM 14232^T, *Pseudoalteromonas mariniglutinosa* DSM 15203^T, and *Pseudoalteromonas neustonica* JCM 31286^T.

Regarding chemotaxonomic analysis, studies of respiratory quinones, polar lipids and fatty acids were performed as follows: Respiratory quinones were extracted from freeze-dried biomass using aqueous methanol and petroleum ether [22]. Then, quinones were separated by thin-layer chromatography (TLC) in a chromatography tank containing petroleum ether and diethyleter (85: 15 (v/v)) [22] and identified by HPLC [23]. Polar lipids extraction was also performed from freeze-dried biomass using aqueous methanol and petroleum ether [22] and then, different polar lipids groups were separated using 2D-TLC [22]. For the detection of polar lipids composition, TLC plates were sprayed using molibdatophosphoric acid, ninhydrin, molybdenum blue, and α-napthol [24, 25]. Finally, fatty acids extracted from 40 mg of bacterial biomass grown on TSA supplemented with 2.5% NaCl (w/v) for 24 h at 28ºC following the protocol outlined by Sasser [26]. Extracted fatty acids were identified using the Microbial Identification System (MIDI) Sherlock Version 6.1 (RTSBA6 database). Fatty acids from the previously mentioned reference strains also were extracted in parallel experiments at the same growth conditions.

Genomic DNA was extracted using a G-spin^{TM} Total DNA Extraction kit (Intron Biotechnology Ltd., Korea) according to the manufacturer's instructions. 16S rRNA gene was amplified as described in Navarro-Torre *et al*. [15]. Partial 16S rRNA gene sequence (1389 bp) was deposited in GenBank/EMBL/DDBJ data library under accession number KU588400 and aligned with corresponding sequences of closely related type strains retrieved by Ez-Taxon-e service (http://www.ezbiocloud.net/eztaxon) [27]. 16S rRNA gene pairwise sequence similarities were determined using the method described by Meier-Kolthoff *et al.* [28]. The phylogenetic tree was inferred using the GGCD web server (http://ggdc.dsmz.de/) [29] according to Montero-Calasanz *et al*. [30]. Draft genome was sequenced using Illumina technology and a standard analysis pipeline by MicrobesNG company (Birmingham, United Kingdom). The closest available reference was identified by Kraken [31]. Quality of data was estimated mapping the reads using BWA mem [32] then, *de novo* assembly was done with SPAdes [33] and again using BWA mem to get more quality metrics. The whole draft genome was deposited in GenBank/EMBL/DDBJ. Finally, the genome annotation and basics statistics was

performed using RAST server v2.0 [34], QUAST v.4.6.3 software [35], PROKKA [36], SignalP 4.1 server [37], TMHMM server v.2.0 [38], and CRISPRFinder [39]. Overall genome related indexes (OGRI) were calculated using GGCD web server [29] (http://ggdc.dsmz.de/) for the digital DNA-DNA hybridization (dDDH) test and JSpeciesWS server [40] (http://jspecies.ribohost.com/jspeciesws) for the average nucleotide identify (ANI) test.

Cells of strain RA15^T were Gram staining-negative, non-spore forming, non-motile, aerobic rods of 2.1 x 1.3 µm (Supplementary Fig. S1). Cells appeared single or in pairs under optical microscope. Although most species described in the genus *Pseudoalteromonas* are motile [1,4,7,8,12,41,42,43], the absence of motility is not exclusive of strain RA15^T as this characteristic was already noted in other species such as *Pseudoalteromonas gelatinilytica* [44]. RA15^T cells formed cream-coloured (RAL 075 90 20), opaque, mucoid, viscous, convex, irregular colonies with an undulate margin and 3.75 mm in size after 24 h on TSA 2.5% NaCl (w/v) plates at 28ºC. Strain RA15^T grew on TSA 2.5% NaCl (w/v) in a range of temperature from 4ºC to 32ºC observing the optimal range from 20ºC to 28ºC (Table 1). Range of pH was from 5.0 to 9.0 with an optimal pH at 7.0-8.0. The tolerance to NaCl was from 0% to 15% (optima growth at 2.5%), but the growth in absence of NaCl was weak (Table 1). These features were very similar with the other species of the genus and according to the genus description [1]. Strain RA15^T also was able to grow on MA as other species described in the genus [1, 4, 7, 8, 12, 44]. Contrarily, growth on MacConkey agar and Cetrimide agar was not observed.

According to Navarro-Torre *et al*. [15], strain RA15^T is positive for the hydrolysis of starch, casein, tween 80, DNA, chitin and pectin. Here, positive results for gelatine and aesculin hydrolysis were also observed (Table 1). The ability of hydrolysing both tween 80 and gelatine is besides in agreement with the emended description of the genus by Ivanova *et al*. [4]. On the other hand, results from API ZYM, API 20NE and API 20Strep galleries reported the presence of alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsine, acid phosphatase, naphthol-AS-BI-phosphophohydrolase, *α*-glucosidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminadase, arginine dihydrolase, pyrolidonyl arylamidase and leucine aminopeptidase (Table 1). Esterase (C4), cysteine arylamidase, *α*-chymotrypsin and *α*-galactosidase activity were

also identified, but it was weak (Table 1). The presence of some enzymes like *β*-glucosidase, cysteine arylamidase, *α*-chymotrypsin, *α*-galactosidase, pyrolidonyl arylamidase and leucine aminopeptidase makes strain RA15^T metabolically different than other species of genus *Pseudoalteromonas* [7, 8, 42, 43]. In addition, API 20NE gallery showed that strain RA15^T reduced nitrates to nitrites and was able to assimilate D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid and malic acid (Table 1). Furthermore, RA15^T was Voges-Proskauer positive and produced the media acidification from D-ribose, D-trehalose, starch and glycogen according to API 20Strep gallery results. These results showed some differences with other species of genus *Pseudoalteromonas* regarding the assimilation of adipic acid and the production of acetoin (Voges-Proskauer positive) [7, 8, 42, 43]. Finally, strain RA15^T was catalase and oxidase positive in line with other species in the genus [1]. Concerning results from Biolog System, strain RA15^T was able to oxidise dextrin, D-maltose, D-trehalose, D-cellobiose, *β*-gentiobiose, sucrose, *N*-acetyl-D-glucosamine, *N*-acetyl-*β*-D-mannosamine, *N*-acetyl-D-galactosamine, D-glucose, D-fructose, D-galactose, L-fucose, L-rhamnose, inosine, 1% sodium lactate, fusidic acid, D-serine, D-mannitol, D-glucose-6-phosphate, D-fructose-6-phosphate, gelatin, glycine-proline, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, L-serine, pectin, D-galacturonic acid, L-galactonic acid-*γ*-lactone, D-gluconic acid, L-malic acid, tween 40, *α*-keto-butyric acid, acetoacetic acid, propionic acid and acetic acid (Table 1). Moreover, strain RA15^T tolerated the presence of rifamycin SV, tetrazolium violet, tetrazolium blue and potassium tellurite (Table 1). Strain RA15^T showed features in common with the closely related species but also some differences (Table 1)

The major respiratory quinones were Q8 (65.8%) and Q9 (11.6%) in keeping with data for other species of *Pseudoalteromonas* [7, 43, 44] and according with the family description [2]. Polar lipids profile consisted of phosphatidylglycerol and phosphatidylethanolamine (Fig. 1) as other species of the genus [7, 42, 44] and two unidentified aminolipids. The major fatty acids were summed feature 3 ($C_{16:1}$ ω7c and/or $C_{16:1}$ *ω*6c; 35.3%), $C_{17:1}$ *ω*8c (16.7%), and $C_{16:0}$ (12.2%) (Table 2).

Phylogenetic analyses affiliated strain RA15^T to the genus *Pseudoalteromonas* forming a well-supported group with the type strains of *P. neustonica, P. prydzensis* and *P. mariniglutinosa* (Fig. 2). 16S rRNA gene sequence similarities with *P. neustonica* JCM 31286^T (98.5%), *P. prydzensis* MB8-11^T (98.1%), and *P. mariniglutinosa* KMM 3635^T

(97.8%) were nevertheless below the 98.7% threshold recommended [28, 45] to confirm the species novelty in the phylum *Proteobacteria*. OGRI tests performed with the genome drafts of *P. neustonica* PAMC 28425^T (accession number BDDS01), *P. prydzensis* MB8-11^T (accession number BDDT01) and *P. mariniglutinosa* KMM 3635^T (accession number BDDU01) (Table 3) supporting the taxonomic allocation of strain RA15^T as new representative of genus *Pseudoalteromonas* [28, 45].

Following proposed minimal standards for the use of genome data for the taxonomy of prokaryotes [45], the whole genome sequence of strain RA15^T, whose accession number is CABVLM01, has a total length of 5,267,131 bp, and is formed for 97 contigs. The N50 values is 328,874, the coverage is 36.7X and the genomic G+C content of 40.4% (Supplementary Table S1).

Combined phenotypic and phylogenetic data demonstrated that RA15^T represents a novel specie in the genus *Pseudoalteromonas*, and the name *Pseudoalteromonas rhizosphaerae* sp. nov. is proposed.

Description of *Pseudoalteromonas rhizosphaerae* sp. nov.

Pseudoalteromonas rhizosphaerae (rhi.zo.sphae′rae. N.L. gen. n. *rhizosphaerae* of the rhizosphere).

Cells are Gram staining-negative, non-spore forming, aerobic rods appearing alone or in pairs. Cells form cream-coloured (RAL 075 90 20), opaque, mucoid, viscous, convex, irregular colonies with an undulate margin of 3.75 mm after 24 h on TSA 2.5% NaCl (w/v) plates at 28ºC (optimal growth conditions). It grows at 4ºC-32ºC and pH 5.0-9.0 and tolerates 0%-15% NaCl (w/v). Grows well on MA, but not on MacConkey agar and Cetrimide agar. Catalase and oxidase positive. Starch, casein, tween 80, DNA, chitin, pectin, gelatine and aesculin are hydrolysed. Strong enzymatic activity is observed for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsine, acid phosphatase, naphthol-AS-BI-phosphophohydrolase, *α*-glucosidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminadase, arginine dihydrolase, pyrolidonyl arylamidase and leucine aminopeptidase, weak for esterase (C4), cysteine arylamidase, *α*-chymotrypsin and *α*-galactosidase, and negative for alkaline phosphatase, lipase (C14), *β*-galactosidase, *β*-glucoronidase, *α*-mannosidase and *α*-fucosidase. According API 20NE, can reduce nitrates to nitrites and is able to assimilate D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid

and malic acid, but cannot assimilate capric acid, trisodium citrate and phenylacetic acid, ferment glucose or produce indole. Positive for production of acetone (Voges-Proskauer positive). Acid is formed from D-ribose, D-trehalose, starch and glycogen, but not from L-arabinose, D-mannitol, D-sorbitol, D-lactose, inulin and D- raffinose. According to Biolog System, positive for the oxidation of dextrin, D-maltose, D-trehalose, D-cellobiose, *β*-gentiobiose, sucrose, *N*-acetyl-D-glucosamine, *N*-acetyl-*β*-D-mannosamine, *N*-acetyl-D-galactosamine, D-glucose, D-fructose, D-galactose, L-fucose, L-rhamnose, inosine, 1% sodium lactate, fusidic acid, D-serine, D-mannitol, D-glucose-6-phosphate, D-fructose-6-phosphate, gelatin, glycine-proline, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, L-serine, pectin, D-galacturonic acid, L-galactonic acid-*γ*-lactone, D-gluconic acid, L-malic acid, tween 40, *α*-keto-butyric acid, acetoacetic acid, propionic acid and acetic acid, but negative for turanose, stachyose, D-raffinose, *α*-D-lactose, D-melibiose, *β*-methyl-D-glucoside, D-salicin, *N*-acetyl-neuraminic acid, D-mannose, 3-*O*-methyl-D-glucose, D-fucose, D-sorbitol, D-arabitol, myo-inositol, glycerol, D-aspartic acid, troleandomycin, minocycline, L-pyroglutamic acid, guanidine hydrochloride, D-glucuronic acid, glucuronamide, mucic acid, quinic acid, D-saccharic acid, *p*-hydroxy-phenylacetic acid, methyl pyruvate, D-lactic acid methyl ester, L-lactic acid, citric acid, *α*-keto-glutaric acid, D-malic acid, bromo-succinic acid, *γ*-amino-*n*-butyric acid, *α*-hydroxy-butyric acid, *β*-hydroxy-butyric acid, sodium formate, butyric acid and sodium bromate. Can grow in presence of rifamycin SV, tetrazolium violet, tetrazolium blue and potassium tellurite but not grow in presence of aztreonam, nalidixic acid, lithium chloride vancomycin lincomycin and niaproof. The major fatty acids are summed feature 3 ($C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c), $C_{17:1}$ ω8c and $C_{16:0}$. The predominant respiratory quinones are Q8 and Q9. Polar lipids profile consists of phosphatidylglycerol, phosphatidylethanolamine and two unidentified aminolipids. The whole genome has a total length of 5,267,131 bp and is formed for 97 contigs. The N50 value is 328,874 and the coverage is of 36.7X. The genomic G+C content is 40.4%.

The type strain, RA15^T (=CECT 9079^T=LMG 29860^T), was isolated from the rhizosphere of the halophyte plant *Arthrocnemum macrostachyum*. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence is KU588400. The GenBank/EMBL/DDBJ accession number for the draft genome is CABVLM01.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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Fig. 1. Total lipid profiles (labelled by the Rf values) of strain RA15^T after separation by two-dimensional TLC using the solvents chloroform/methanol/water (65 : 25 : 4, by vol.)

in the first dimension, and chloroform/methanol/acetic acid/water (80 : 12 : 15 : 4, by vol.) in the second dimension. Plates were sprayed with molybdatophosphoric acid (3.5 %; Merck) for detection of total polar lipids. PE, phosphatidylethanolamine; PG, phosphatidylglycerol; AL1-2, unidentified aminolipid.

Fig. 2. Maximum-likelihood phylogenetic tree inferred from 16S rRNA gene sequences, showing the phylogenetic position of strain RA15^{T} relative to type strains of species within the genus *Pseudoalteromonas*. The branches are scaled in terms of the expected number of substitutions per site. Support values obtained from 1000 replicates from maximum-likelihood (left) and maximum-parsimony (right) bootstrapping are shown above the branches if ≥60%. Sequence accession numbers are given in parentheses.

Table 1. Differential characteristics of strains RA15^T and the closely strains of the genus *Pseudoalteromonas*.

Strains: 1, Strain RA15^T; 2, *P. prydzensis* ACAM 620^T; 3, *P. mariniglutinosa* NCIMB 1770^T ; 4, *P. neustonica* JCM 31286^T. +, positive; -, negative ; w, weak ; ND, data no available.

Characteristics	**1**	**2**	**3**	**4**
NaCl range (%, w/v)	0-15	0.5-15*[a]	1-9*[b]	1-7*[c]
Temperature (ºC) Range	4-32	0-30*[a]	5-37*[b]	4-30*[c]
Optimum	20-28	22-25	20-28*[b]	25*[c]
Hydrolysis of:				
Aesculin	+	+*[a]	+*[b]	-*[c]
Production of:				
Esterase lipase (C8)	+	ND	w*[b]	-*[c]
Valine arylamidase	+	ND	-*[b]	-*[c]
Trypsine	+	ND	-*[b]	-*[c]
α-glucosidase	+	ND	-*[b]	-*[c]
Arginine dihydrolase	+	-*[a]	-*[b]	-*[c]
Reduction nitrates to nitrites	+	-*[a]	-*[b]	-*[c]
Acid production from:				
D-mannitol	-	-*[a]	+*[b]	ND
Oxidation of:				
D-raffinose	-	+	+	ND
D.fructose	+	+	-	ND
D-galactose	+	-	-	ND
L-rhamnose	+	-	+	ND
D-glucuronic acid	-	+	+	ND
Susceptibility to:				ND
Rifamycin SV	+	-	-	ND
Tetrazolium violet	+	-	-	ND
Tetrazolium blue	+	-	-	ND
Lithium chloride	-	-	+	ND
Aztreonam	-	-	+	ND

[a] Data from Bowman *et al*. [41].
[b] Data from Romanenko *et al.* [42].
[c] Data from Hwang *et al*. [8].

Table 2. Cellular fatty acid compositions (%) of RA15^T and closely related *Pseudoalteromonas* species.

Strains: 1, RA15^T; 2, *P. prydzensis* DSM 14232^T; 3, *P. mariniglutinosa* DSM 15203^T; 4, *P. neustonica* JCM 31286^T. -, not detected; tr, values below 1%. Values below 1% in all columns are not displayed. All data are obtained in this study.

Fatty acid	1	2	3	4
$C_{11:0}$ 3-OH	2.4	1.1	2.2	-
$C_{12:0}$	2.7	2.2	2.3	tr
iso-$C_{12:0}$ 3-OH	1.7	1.6	1.9	-
$C_{12:0}$ 3-OH	5.2	6.3	6.4	-
$C_{14:0}$	1.0	2.2	1.5	5.6
$C_{15:1}$ ω8c	4.7	3.4	2.5	tr
Summed feature 3[a]	35.3	38.7	33.3	-
$C_{16:0}$	12.2	20.8	16.7	46.2
iso-$C_{16:0}$	tr	1.6	1.3	-
$C_{17:1}$ ω8	16.7	7.9	11.8	-
anteiso-$C_{17:0}$	tr	1.2	1.2	-
$C_{17:0}$	4.5	2.7	3.7	-
$C_{18:0}$	tr	tr	1.1	1.4
$C_{18:1}$ ω9c	tr	tr	1.4	38.5
10-methyl $C_{18:0}$	-	-	-	4.6
Summed feature 8[b]	6.4	4.7	4.7	-
Summed feature 7[c]	tr	tr	4.7	tr

*Summed features are groups of 2 or 3 fatty acids that are treated together for the purpose of evaluation in the MIDI System and include both peaks with discrete equivalent chain-lengths (ECLs) as well as those where the ECLs are not reported separately [46].

[a] Summed feature 3 was listed as $C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c.

[b] Summed feature 8 was listed as $C_{18:1}$ ω7c and/or $C_{18:1}$ ω6c.

[c] Summed feature 7 was listed as $C_{19:1}$ ω7c and/or $C_{19:1}$ ω6c

Table 3. Overall genome related index (OGRI) of strain RA15^T.

Strains	dDDH	ANIb	ANIm
P. neustonica PAMC 28425^T	24.50%	80.54%	85.45%
P. prydzensis MB8-11^T	28.20%	83.93%	86.78%
P. mariniglutinosa KMM 3635^T	25.80%	82.11%	85.45%